COMPARATIVE CARBOHYDRATE PHYSIOLOGY OF ECTO- AND ENDOMYCORRHIZAS

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INTRODUCTION

No detailed studies on the carbohydrate physiology of vesicular-arbuscular mycorrhizas have yet been made, although carbohydrate levels in mycorrhizal and non-mycorrhizal plants under a range of environmental and nutritional conditions have been reported (Bevege, 1971; Hayman, 1974) and Ho and Trappe (1973) recorded the transfer of ^{14}C compounds from the higher plant to mycelium of *Endogone*. Although rather more studies have been made on ectomycorrhizas, the most detailed are those of Lewis and Harley (1965) with excised *Fagus sylvestris* mycorrhizas fed with ^{14}C-labelled sugars. Melin and Nilsson (1957) demonstrated the transfer of ^{14}C assimilate from *Pinus sylvestris* to the associated mycorrhizal fungus under axenic conditions. Nelson (1964), Shiroya *et al.*, (1962), Lister *et al.*, (1968) and Schweers

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and Meyer (1970) presented evidence that ectomycorrhizas of *P. resinosa, P. strobus* and *P. sylvestris* could act as sinks for photosynthates in plants exposed to $^{14}CO_2$ but the detailed chemical fate of the carbohydrate in these systems was not examined. In *Fagus* mycorrhizas, sucrose transferred to the fungus is stored principally as trehalose and mannitol, thus maintaining a concentration gradient for further movement of sucrose (Lewis & Harley, 1965).

In view of the fact that polyols have not yet been demonstrated as occurring in Mucorales, it was appropriate that we examine the fate of photosynthesized $^{14}CO_2$ in endomycorrhizal plants, specifically enquiring into the chemical partitioning of assimilate translocated to the fungus, and whether vesicular-arbuscular mycorrhizas act as significant assimilate sinks. It was also appropriate to extend the detail of the Lewis and Harley studies to *Pinus*, using intact plants assimilating $^{14}CO_2$. This paper therefore reports the fate of ^{14}C-labelled photosynthate in endomycorrhizal hoop pine (*Araucaria cunninghamii* Ait.) and subterranean clover (*Trifolium subterraneum* L. var. Bacchus March), comparing their carbohydrate physiology with that of ectomycorrhizal radiata pine (*Pinus radiata* D. Don). Information is also presented on the pattern of assimilation in *Endogone*.

METHODS

Plant Growth

Pilot studies with hoop pine were conducted on 18 months old pot plants raised in organic amended nursery soil infected with a mixture of *Endogone araucareae* (Bevege 1971), *E. mosseae* and *E. macrocarpa*. Otherwise, 40 months old seedlings were raised from surface-sterilised seed in steam-sterilised peat/sand mix with complete nutrient solution

and inoculated with spores of *E. araucareae*; control plants received spore washings to ensure a common microflora. The plants were grown in 100 g of medium in 20 x 3 cm cotton-wool plugged glass tubes.

Radiata pine raised from surface-sterilised seed were grown for six months in gamma-irradiated (2.5 Mrad) Mt. Burr sand (Stephens *et al.*, 1941) inoculated with *Rhizopogon luteolus*. The plants were grown in porcelain enamelled pots capacity 4 kg, with four seedlings/pot, and fertilised with 30 kg ha^{-1} superphosphate.

Clover plants from surface-sterilised seed were grown for 52 days in gamma-irradiated Wanneroo sand with complete nutrient solution (10 ml/plant of half strength complete nutrient solution, Hoagland & Arnon, 1938). Inoculation with *E. mosseae* was made on day 8 and again on day 22; control plants received spore washings only. Containers were 2.5 cm diameter polythene tubes of 250 g medium capacity with one plant/tube.

Hoop and radiata pines were raised under glasshouse conditions and the clover in a controlled environment, 18 hr day (20 400 lumen m^{-2})/6 hr night at 20°C/15°C respectively. Labelling of clover was conducted under the same conditions and that of hoop and radiata pines was under 16 hr day (20 400 lumen m^{-2})/8 hr night at a constant 20° and 22°C ± 1°C respectively.

$^{14}CO_2$ Labelling

Shoots were isolated from roots by a mineral oil/wax barrier (melting point 42°C) poured on to the surface of the soil and sealing around the stem. When labelling intermittently over several days, a breather tube to ensure adequate root aeration was inserted in the soil prior to preparing the wax barrier; this tube was temporarily sealed during the labelling period each day. At labelling, shoots were enclosed in a polythene bag sealed

around the container and the appropriate volume of $^{14}CO_2$ injected into the enclosed atmosphere with a gas syringe and the hole sealed with cellulose tape. Labelled $^{14}CO_2$ was generated from $Na_2{}^{14}CO_3$ with lactic acid (McDougall & Rovira, 1965) and unless otherwise stated 20 μCi $^{14}CO_2$ per plant was introduced to give a final CO_2 concentration of 1% in a volume of 1 500 ml approximately. Plants were usually exposed to $^{14}CO_2$ for 2-4.5 hr as a single pulse label followed by harvest 18-20 hr later which included a dark period of 6-8 hr. In experiments with labelling over 6 days (see text) daily 2 hr pulses with 20 μCi $^{14}CO_2$ were applied.

Analysis of ^{14}C-Labelled Photosynthate

Spatial distribution of ^{14}C activity in roots was examined by macroautoradiography (Kodirex X-ray film). Roots were carefully washed and *Endogone* hyphae was either completely harvested (clover) or sampled (hoop pine). Selected root material was lightly blotted dry, weighed, plunged into boiling 80% ethanol and extracted four times at 80°C over 20 hr. The ethanol soluble extract was cleared and deionised following the procedures of Somogyi (1945) and Lewis and Harley (1965). Extracts were concentrated to less than 10 ml under reduced pressure at 50°C and brought to 50 ml with distilled water; 2.5 ml carbonate-free $Ba(OH)_2$ was added followed by 2.5 ml 5% $ZnSO_4$ 3 mins later. The resulting precipitate (the metal-precipitated fraction) was filtered off and the now-clear and decolourized filtrate made up to standard volume. Half the filtrate was deionised by adding a 2:1 mixture of 1R-4B(OH): 1R-120(H) Amberlite exchange resin (the neutral soluble fraction). The ethanol insoluble material was hydrolysed with 1.5N H_2SO_4 under reflux for 2 hr, the hydrolysate was then neutralised with $BaCO_3$ and the filtrate deionised as before. Residue was digested with 5N LiOH for 4 hr.

Some further biochemical characterisation was attempted of compounds in the various fractions prepared. The ethanol soluble fraction is recorded as "neutral", "ionised" and "metal-precipitated". The first two fractions are considered to consist mostly of carbohydrates, and of organic and amino acids respectively. The composition of the "metal-precipitated" fraction is more complex and while we consider it to comprise lipids, lipid-protein and some soluble protein (Davies *et al.*, 1964, and helpful discussion with Professor K. J. Scott), further definitive study is obviously needed. Kjeldahl analyses indicated the crude ethanol extracts contained 20-25 % of total root nitrogen, or 0.18% of total root weight and we assume the source of this ethanol soluble nitrogen is predominantly protein and phosphatides as well as amino acids. The neutral sulphuric acid hydrolysate is recorded as polysaccharide, while the residue is considered to contain cell wall material and protein.

All fractions from the above analysis were retained and ^{14}C activity measured by liquid scintillation counting. Precipitates were counted following suspension in thixotropic gel. Scintillation fluid was based on POPOP in toluene and 2 methoxyethanol.

Sugars in the neutral ethanol fraction and in the neutral hydrolysate were separated and putatively identified by one-way paper chromatography. Double runs were made with each of two solvent systems: ethylacetate-acetic acid-water, and n propanol-ethylacetate-water (Lewis & Harley, 1965). Chromatograms were scanned with a radiochromatogram strip scanner (Nuclear Chicago Actigraph III) and relative proportions of various sugars calculated from the respective areas under the trace. Putative identities of sugars were further checked by co-chromatography with known compounds, using two-way thin-layer chromatography on activated boric acid buffered silica gel plates using n butanol-acetic acid-

diethyl ether-water and isopropanol-ethyl acetate-water (Bevege, 1971). Radioactive spots on the plates were located by autoradiography on X-ray film.

SPATIAL DISTRIBUTION OF ^{14}C IN HOOP PINE ROOTS

The root systems of hoop pine had three types of rootlets (a) unsuberized actively growing uninfected laterals, (b) terminal unsuberized infected short roots and (c) suberized infected short roots, either terminal or sub-terminal to root type (b). Autoradiograms (Figure 1) indicated that ^{14}C was concentrated in the unsuberized roots; young mycorrhizas

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Figure 1. Spatial Distribution of ^{14}C in hoop pine roots.

A. Plants 18 months old, mixed inoculum from soil. Two hour exposure to 20 $\mu Ci\ ^{14}CO_2$; harvested 18 hr later.

Note the order of concentration of label in root types is (a) greater than or equal to (b) greater than (c).

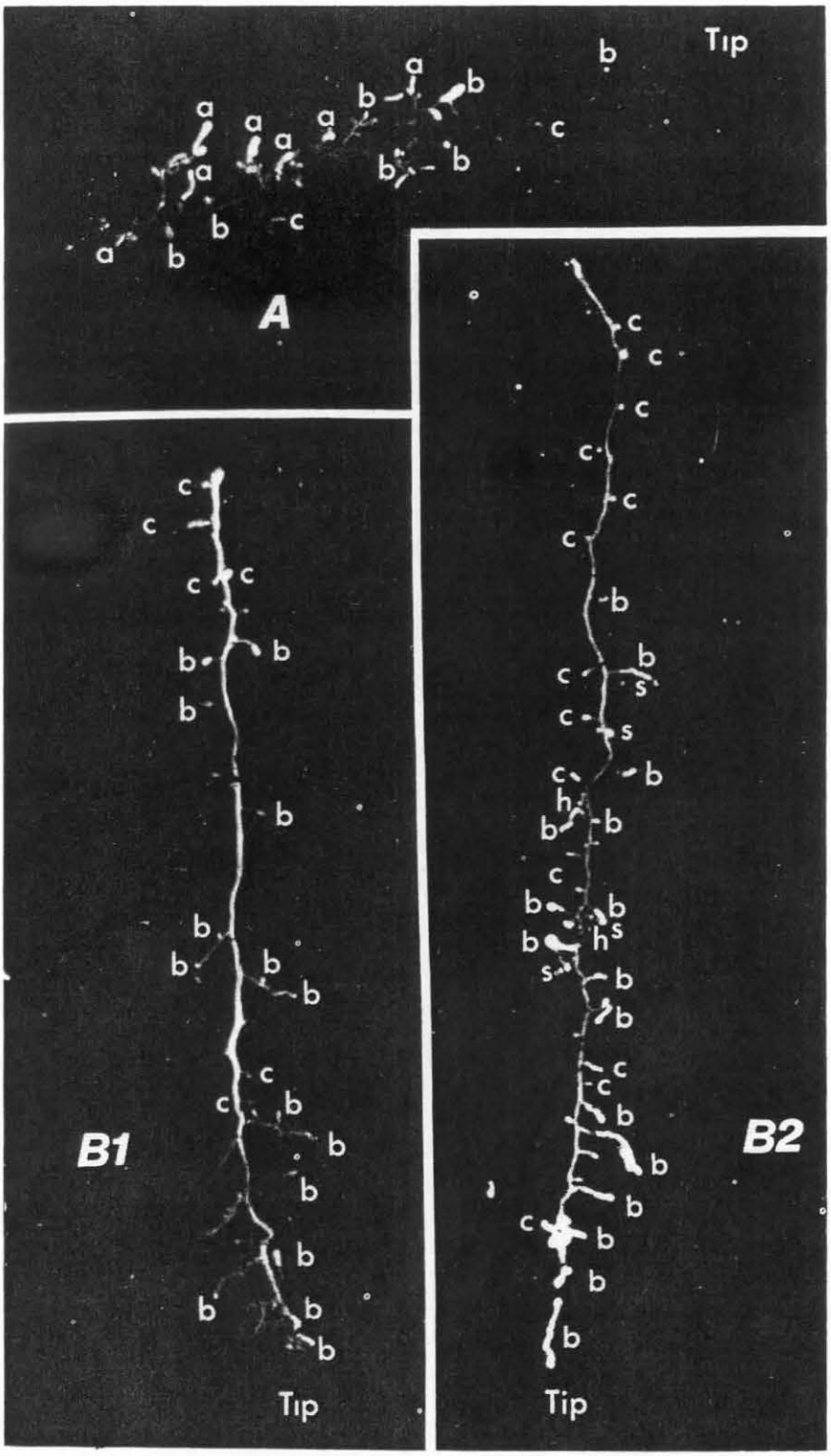
B1. Plants 40 months old, inoculated *Endogone araucareae*. Two hour exposure to 120 $\mu Ci\ ^{14}CO_2$, harvested 24 hr later; 6 day exposure to X-ray film.

B2. Plants 40 months old, inoculated *Endogone araucareae*. Two hour exposure daily for 6 days, harvested 24 hr later; 2 day exposure to X-ray film.

Note concentration of label in type (b) roots is greater than (c) and labelling of extra-matrical hyphae and spores of the endophyte.

Key to lettering:

a. unsuberized actively growing uninfected laterals
b. terminal unsuberized infected short roots
c. suberized infected short roots
h. extra-matrical hyphae
s. zygospores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A
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(type (b)) do not appear to be more active in this regard than actively growing uninfected roots (type (a)) and older suberized mycorrhizas (type (c)) are less active than young mycorrhizas. Type (b) roots are in the active development stage with arbuscules predominating over vesicles (see plate in Bevege, 1968) which in turn tend to predominate in the suberized roots; this pattern of preferential movement of photosynthate to actively metabolising roots is consistent with observations made on wheat roots by Rovira and Bowen (1973).

THE CARBOHYDRATE COMPONENTS OF MYCORRHIZAS

Hoop Pine

Using co-chromatography, Bevege (1971) located putative mannose, arabinose, glucose, galactose, fructose, sucrose and raffinose in the neutral ethanol extract of hoop pine mycorrhizas. As these were also present in foliage, he concluded there was no sugar in roots which might be distinctive for the fungal endophyte. Neither mannitol nor trehalose was found. Hayman (1974) also failed to detect these fungal carbohydrates in endomycorrhizas of onion. Sulphuric acid hydrolysates of the ethanol insoluble fraction of the hoop pine roots studied by Bevege (1971) contained glucose, xylose and galactose while perchloric acid hydrolysis yielded only glucose. He concluded that the main polysaccharides were glucosan and xylan with some galactan present, the latter two probably arising from cell walls (Davies *et al.*, 1964). Histochemical techniques in both light and electron microscopy demonstrated starch in the higher plant and glycogen in *Endogone* hyphae.

In the present study, the neutral ethanol soluble extract of mycorrhizas and uninfected roots contained similar levels of the same sugars in approximately the same proportions (Table 1).

By far the greatest activity was found in sucrose followed by glucose; other sugars were relatively minor components. Arabinose was not detected but was one of the sugar moieties found in the acid hydrolysate along with glucose, xylose and galactose. No polyols, inositols or trehalose were detected. Insufficient material was available to permit determination of carbohydrates in *Endogone* hyphae associated with roots.

Table 1. Percent Composition of Root Sugars of Hoop Pine[1]

Sugar	Mycorrhizas		Uninfected Roots	
	1 day[2] label	6 day[3] label	1 day[2] label	6 day[3] label
Sucrose[4]	66.5	69.0	64.5	61.8
Glucose	16.0	7.2	10.5	16.8
Galactose	9.5	10.2	17.5	6.8
Mannose	5.5	5.8	7.5	7.0
Raffinose	2.5	4.5	0	4.2
Xylose	0	2.5	0	1.8
Fructose	0	0.7	0	1.5
Activity Counts/min/mg	449	4078	431	4398

1. inoculated with *Endogone araucareae*; 2 replications/treatment, sample size 11–27 mg roots for analysis.
2. 24 hr after a 2 hr pulse of 120 µci $^{14}CO_2$/plant.
3. labelled daily for 6 days with 20 µCi $^{14}CO_2$ plant; harvested 24 hr after final pulse.
4. putative identifications based on co-chromatography with known carbohydrates.

Radiata Pine

In parallel investigations to those described above, the sugar composition of radiata pine mycorrhizas (endophyte *R. luteolus*) and uninfected roots on the same plants was examined. The host sugars of uninfected roots were sucrose, glucose and fructose, with sucrose predominating (Table 2). This parallels the observations made with other species of *Pinus* (Shiroya *et al.*, 1962, Schweers & Meyer 1970). In contrast to the uninfected roots, the fungal carbohydrates mannitol and trehalose dominated the mycorrhizas, making up 68% of the ^{14}C assimilate; there were also traces of xylose. This observation parallels the experience of Lewis and Harley (1965) who fed labelled sucrose to excised *Fagus* mycorrhizas with an unidentified endophyte. The polysaccharides contained the moieties glucose, arabinose, xylose and ribose; Lewis and Harley (1965) similarly detected these sugar components in the hydrolysate of *Fagus* mycorrhizas. In the present study we could detect no differences between the polysaccharides of mycorrhizas and uninfected roots. However, if one accepts that the primary polysaccharide of the fungus is glycogen (as suggested by Lewis & Harley) then an increase in the proportion of glucose is all that may be detected following hydrolysis.

The contrast between the carbohydrates of ecto- and endomycorrhizas is marked. The former contained four host-derived sugars plus two fungal carbohydrates, the latter eight sugars in all, none of which could be regarded as exclusively a fungal metabolite. While it is likely that sucrose is the main carbohydrate transferred to the fungus and passive movement of sucrose along a concentration gradient occurs in both systems, it seems this is achieved in quite different ways. The ectomycorrhizas keep sucrose concentration low by converting it to trehalose and mannitol, thus becoming a highly effective sink, but other systems operate with vesicular-arbuscular mycorrhizas.

Table 2. Percent Composition of Root Carbohydrates of Radiata Pine[1]

Carbohydrate	Mycorrhizas		Uninfected Roots[2]	
	1 day[3] label	6 day[4] label	1 day[3] label	6 day[4] label
Sucrose[5]	6.5	7.2	56.0	ND[6]
Glucose	18.5	21.5	34.5	ND
Fructose	5.8	3.9	9.5	ND
Xylose	1.5	0	0	ND
Trehalose	52.2	45.7	0	ND
Mannitol	15.5	21.7	0	ND
Activity Counts/min/mg	79754	15492	3543	1408

1. inoculated with *Rhizopogon luteolus*; 4 replications/treatment, sample size 6 mycorrhizas or rootlets for analysis.
2. roots from same plants as mycorrhizas.
3. 24 hr after a 2 hr pulse of 120 $\mu Ci\ ^{14}CO_2$/plant.
4. labelled daily for 6 days with 20 $\mu Ci\ ^{14}CO_2$/plant; harvested 24 hr after final pulse.
5. putative identifications based on co-chromatography with known carbohydrates.
6. not determined; samples lost during chromatography.

^{14}C INCORPORATION INTO MYCORRHIZAS AND UNINFECTED ROOTS

The incorporation of ^{14}C into roots of three specific systems was investigated (a) radiata pine infected with *R. luteolus*, comprising mycorrhizas and uninfected roots on the same plants, (b) hoop pine infected with *E. araucareae* compared to uninfected

plants of equivalent development and (c) clover infected with *E. mosseae* compared with uninfected plants.

Radiata Pine

Plants were pulse labelled with 20 μCi $^{14}CO_2$ per plant daily for 6 days or with 120 μCi $^{14}CO_2$ per plant on the sixth day and harvested on the seventh day. As Rangnekar and Forward (1972) have pointed out, the pattern of distribution of the ^{14}C-label becomes established within 3 days in *P. resinosa*, so that the difference between 24 hr and 7 day distribution patterns enables a comparison of the rate of metabolic incorporation between mycorrhizal and uninfected plants.

After 24 hr, mycorrhizas had 15 times the activity of uninfected roots, demonstrating considerable capacity to attract photosynthate; 74% of this was in the carbohydrate fraction compared to 51% in uninfected roots (Table 3). The difference represents fungal carbohydrate, as 68% of mycorrhiza carbohydrate was in trehalose and mannitol i.e. 50% of the total label. Only slight proportional differences were found between the levels of other components of the system although uninfected roots tended to have more assimilate converted to structural material, polysaccharide and acid at this time. This suggests translocation to roots in an ectomycorrhizal plant will be greater than in a non-mycorrhizal plant of the same size. This point has yet to be demonstrated, and it is unfortunate that the data of Nelson (1964), while undoubtedly indicating increased translocation to roots of mycorrhizal plants, is based on plants of different sizes and root/shoot ratios.

After 7 days, mycorrhizas still had 8 times the activity of other roots but the difference in the proportions in the respective carbohydrate pools had narrowed; fungal carbohydrate still comprised 67% of total soluble in mycorrhizas with mannitol

Table 3. Fractionation of Assimilate of Radiata Pine[1] (counts/min/mg fresh wt.)

Fraction	Mycorrhizas		Uninfected Roots[2]	
	1 day[3] label	6 day[4] label	1 day[3] label	6 day[4] label
EtoH Soluble				
Metal-Precipitated	3904(3.6)	3618(11.6)	540(7.7)	562(15.6)
Ionised	757(0.7)	410(1.3)	632(9.0)	587(16.2)
Neutral	79754(74.3)	15492(49.6)	3544(50.6)	1408(39.0)
H_2SO_4 hydrolysable				
Neutral	17935(16.7)	8100(25.9)	1447(20.7)	644(17.8)
Ionised	472(0.4)	1029(3.3)	0(0)	0(0)
Residue	4450(4.1)	2592(8.3)	841(12.0)	408(11.3)
Total	107271(100)	31240(100)	7004(100)	3610(100)
Fresh Wt. (mg) of 6 rootlets	1.91		1.04	

1. inoculated with *Rhizopogon luteolus*; 4 replications/treatment, sample size 6 mycorrhizas or rootlets for analysis. Percentage of total ^{14}C in brackets.
2. roots from same plants as mycorrhizas.
3. 24 hr after a 2 hr pulse of 120 $\mu Ci\ ^{14}CO_2$/plant.
4. labelled daily for 6 days with 20 $\mu Ci\ ^{14}CO_2$/plant; harvested 24 hr after final pulse.

increasing relative to trehalose. Uninfected roots maintained a high proportion of their assimilate in non-carbohydrate pools but mycorrhizas retained relatively more in their polysaccharide pool. The reduction in activity over seven days was probably due to respiration loss; Ursino and Paul (1973) measured a 40% respiration loss of ^{14}C-assimilate within 48 hr, in *P. strobus*. In the present study losses from mycorrhizas were 70% (derived principally from the carbohydrate and glycogen fractions) and 50% from uninfected roots, indicating a higher metabolic rate in the former than the latter; Schweers and Meyer (1970) also found respiration increased in mycorrhizas of *P. sylvestris* compared to uninfected roots.

Hoop Pine

Plants were pulse labelled as for radiata pine. After 24 hr, activity in mycorrhizas was no greater than that of uninfected roots and both categories had over 80% of the ^{14}C activity in the soluble carbohydrate fraction, i.e. about 53% of the total label in sucrose. After 7 days, overall activity had increased 11-fold in both kinds of roots indicating that respiratory loss of assimilate was small over the period (Table 4) especially when compared with ectomycorrhizas of radiata pine. This result reflects the relatively slow metabolic rate of hoop pine compared to radiata pine. Increased activity after 7 days in hoop pine was measured in all pools, but was mainly in the soluble carbohydrate fraction (74-77%) with some proportional increase in the polysaccharide and cell wall/protein pools; there was little change in the metal-precipitated fraction and a slight drop in the proportion of acids.

The striking feature is the close similarity between the relative sizes of the various pools in mycorrhizas and uninfected roots, from which we conclude that there is little physiological differ-

Table 4. Fractionation of Assimilate of Hoop Pine and Endogone[1] (Counts/min/mg fresh wt.)

	Mycorrhizas		Uninfected Roots		Endogone Hyphae[4]	
	1 day label[2]	6 day label[3]	1 day label[2]	6 day label[3]	1 day label[2]	6 day label[3]
EtoH soluble						
Metal-Precipitated	31(6.1)	429(7.0)	25(4.9)	334(5.5)	969(48.7)	2799(39.8)
Ionised	28(5.4)	80(1.5)	33(6.4)	140(2.4)	634(31.2)	862(12.2)
Neutral	449(82.2)	4078(74.1)	431(83.8)	4398(77.7)	290(14.6)	530(7.5)
H_2SO_4 hydrolysable						
Neutral	13(2.6)	627(10.0)	14(2.7)	441(7.5)	0(0)	835(11.9)
Ionised	5(0.9)	20(0.2)	2(0.4)	19(0.3)	0(0)	35(0.5)
Residue	14(2.7)	448(7.1)	9(1.8)	394(6.6)	107(5.4)	1980(28.1)
Total	540(100)	5683(100)	513(100)	5725(100)	2000(100)	7041(100)
Fresh Wt. (mg) of sample	27.2	18.5	16.9	10.8	NOT DETERMINED	

1. inoculated with *Endogone araucareae*; 2 replications/treatment. Percentage composition in brackets.
2. 24 hr after a 2 hr pulse of 120 $\mu Ci\ ^{14}CO_2$/plant.
3. labelled daily for 6 days with 20 $\mu Ci\ ^{14}CO_2$/plant; harvested 24 hr after final pulse.
4. hyphal counts are for the total sample.

entiation between the two kinds of root. Furthermore, as uninfected and mycorrhizal plants were the same size, there is no evidence for mycorrhizas influencing the rate or pattern of translocation. This conclusion is supported by the data of Table 1 which shows that both kinds of roots have an identical suite of sugars, there being slight differences only in the relative proportions of glucose and sucrose. The very striking differences in labelling patterns between the plant roots and the extramatrical hyphae shown in Table 4 are discussed below.

Clover

Plants were inoculated at a specific point on the root system and at the time of harvest systemic infection was restricted to this inoculated zone. In the discussion below, the mycorrhizal roots are those from this zone while uninfected roots comprise those from the corresponding zone of uninoculated plants. Total fresh weight and ^{14}C activity were determined for roots from the inoculation zone (the subtending roots), the remaining root system, shoots and *Endogone* hyphae external to the infected subtending roots (extramatrical hyphae). There was extensive proliferation of roots and hyphae over the experimental period. The weight of subtending roots was 14% of the total root weight and that of extramatrical hyphae 6% of subtending root weight.

Plants were exposed to $^{14}CO_2$ for 4.5 hr followed by 18.5 hr assimilation time. Activity was assessed in the ethanol soluble and acid hydrolysable fractions only, as the hoop pine studies indicated the label in the residue fraction was quite small (less than 3%) after 24 hr. Only 6% and 12% respectively of measured activity was located in the soluble carbohydrate pools of mycorrhizas and uninfected roots, whereas the incorporation into the metal-precipitated and polysaccharide fractions together accounted for 90% and 76% respectively.

Table 5. Fractionation of Assimilate of Clover[1]

Fraction	Mycorrhizas count/ min/mg	Mycorrhizas total[2] count	Mycorrhizas per-cent	Uninfected Roots count/ min/mg	Uninfected Roots total count	Uninfected Roots per-cent	Endogone Hyphae count/ min/mg	Endogone Hyphae total count	Endogone Hyphae per-cent
EtOH soluble									
Metal-precipitated	743	440	(71.9)	984	116	(52.4)	2101	84	(56.2)
Ionised	7	3	(0.5)	35	4	(2.0)	220	9	(5.9)
Neutral	64	35	(5.7)	237	27	(12.5)	296	12	(7.9)
H_2SO_4 hydrolysable									
Neutral	204	121[3]	(19.8)	423	43	(19.6)	704	28	(18.8)
Ionised	23	13	(2.1)	173	30	(13.5)	419	17	(11.2)
Total	1042	612	(100)	1853	220	(100)	3741	150	(100)

1. inoculated with *Endogone mosseae*; labelled 18 hr prior to harvest with 20 $\mu Ci\ ^{14}CO_2$/plant. 5 replications/treatment. Percentage composition in brackets.

2. Total activity in fractions measured (counts/min $x10^{-3}$) for the total weight of roots or hyphae in the inoculated zone and the corresponding zone in controls.

3. differences mycorrhizas vs uninfected significant at 10 percent level.

This pattern differs markedly from that of hoop pine where the carbohydrate component made up 80% of activity. Differences are probably due to variation in host physiology; clover is a more vigorously growing species than hoop pine and would therefore be expected to incorporate assimilate more rapidly. The polysaccharide of mycorrhizas and uninfected roots did not exhibit very great differences on a proportional basis although in terms of total activity roots from mycorrhizal plants had nearly 3 times the ^{14}C level of roots from uninfected plants; this however was a consequence of the greater weight of roots as mycorrhizal plants were much larger than uninfected ones (Table 6).

Endogone

Endogone araucareae hyphae were also fractionated after 24 hr and 7 days (Table 4). While the total counts for each sample cannot be compared directly because hyphal weight was not recorded, the proportions of ^{14}C-assimilate in the various pools are of interest. After 24 hr the metal precipitated and ionised pools contained 80% of the assimilate between them and the soluble carbohydrate pool only 15%; there was no transfer at this stage into glycogen. After 7 days, these fractions still accounted for 52% of the assimilate, the decrease being accounted for to some extent by a relative increase in the cell wall plus protein fraction. Glycogen increased and the proportion of soluble carbohydrates decreased. Comparing the soluble carbohydrates of mycorrhizas and hyphae, it is evident that the high level of sugars in the mycorrhizas was not matched by a corresponding level in the external hyphae. After 7 days, host polysaccharide (predominately starch) and that of hyphae (i.e. glycogen) both comprised about 10% of their respective labels. The big difference in the relative proportions of carbohydrate and non-carbohydrate between host and

Table 6. Effect of Mycorrhizas on Growth and Assimilation of $^{14}CO_2$ by Clover[1].

Plant Part	Mycorrhizas	Uninfected Roots	Significance[2]
(a) *Fresh Wt.* (g)			
Tops	0.21 ± 0.05	0.14 ± 0.02	NS
Roots	4.47 ± 0.69	1.77 ± 0.20	*
Total Plant	4.68 ± 0.71	1.91 ± 0.20	*
Hyphae	0.04 ± 0.01	——	
(b) *Total Activity*[3] (counts/min X10^{-3})			
Tops	9756 ± 996	7110 ± 756	
Roots	3294 ± 468	2010 ± 198	(*)
Total Plant	13050 ± 1320	9120 ± 960	(*)
Hyphae	150 ± 42	——	

1. Plants 52 days old inoculated with *Endogone mosseae* on days 8 and 22; labelled 18 hr prior to harvest with 20 μCi $^{14}CO_2$/plant. 5 replications/treatment. Means ± standard error.
2. NS not significant; (*) difference significant at 10 percent level; * difference significant at 5 percent level.
3. Activity in ethanol-soluble and H_2SO_4-hydrolysable fractions; no counts were made on residues. See text.

external hyphae is evidence for the relatively small amount of hyphae present within the root.

Hyphae of *E. mosseae*, after an assimilation period of 18.5 hr, incorporated assimilate into metal-precipitated and polysaccharide pools (56% and 19% respectively). The 8% in the soluble carbohydrate pool corresponded to the 6% in the equivalent pool in the mycorrhizas (Table 5) and there was also a close correspondence between the relative sizes of the polysaccharide pools (18% and 20% respectively). The clover plant and fungus therefore incorporated assimilate in the same manner.

Although both species of *Endogone* diverted a high proportion of assimilate in 24 hr to metal-precipitable material, *E. araucareae* had a relatively high label in the ionisable fraction but not in polysaccharides, but the reverse occurred in *E. mosseae*. Whether these differences between species are conditioned by host physiology or are due to the age and state of the mycelium is unknown; as both fungi form mycorrhizas with clover (Bevege & Bowen, 1975), a study of their relative carbohydrate physiology when growing in conjunction with a common host is worthy of further study.

CONCLUSION

The concept of ectomycorrhizas acting as a physiological sink for assimilate is well established (Harley & Lewis, 1969). Lewis and Harley (1965) elucidated the mechanism of the transfer from host to fungus in *Fagus sylvatica* and our studies confirm this for the *Pinus radiata* - *Rhizopogon luteolus* ectomycorrhiza (see Table 2) namely the one-way transfer of carbohydrate from host to endophyte where it is converted to specific fungal carbohydrates, trehalose and mannitol, and ultimately the storage polysaccharide glycogen, none of which can be utilised by the host plant. In this way a concentration

gradient of sugar, predominantly sucrose, is maintained from host to endophyte. Smith *et al.*, (1969) pointed out the generality of this in many host-parasite relations of plants and Jankiewicz *et al.*, (1969) discussed similar phenomena involving insect parasites.

The storage of carbohydrates in vesicular-arbuscular mycorrhizas appears to be small: trehalose and mannitol or other polyols were not found. Some storage occurred as glycogen. The high proportion in the metal-precipitated fraction, which probably contains lipid, lipo-protein and soluble protein, needs further study, and is consistent with the suggestion of Cox *et al.*, (1975) that lipid may serve an important storage function in these mycorrhizas. In *E. araucareae*, the large label in the ionised fraction containing organic and amino acids (at 1 day) and the great increase in label in the cell wall plus protein fraction over 6 days suggests a significant part of the assimilate "sink" is related to growth of hyphae rather than storage.

The diversion of assimilate to a "growth" sink will be determined by conditions for mycelial growth. Such a sink would be far less than the "storage" sink (and growth sink) in ectomycorrhizas. However, diversion of assimilate by this means can be quite significant. In clover, where infection was deliberately restricted to a localised part of the root system (see above), activity per unit weight of hyphae was nearly 4 times, and total activity 24%, that of subtending roots. A removal of this magnitude from the entire root system of a systemically infected plant plus some respiratory loss by hyphae would represent a considerable drain on host carbohydrate supply; in the present instance, the localised removal by hyphae represented only 1% of total root weight and 4% of total root activity (Table 6). In hoop pine the relative amount of hyphae was very much less. Obviously wide differences in hyphal production occur (see also Bevege &

Bowen, 1975) and this aspect is deserving of much closer study.

The extramatrical *Endogone* hyphae in the clover experiment was only 1% of the total weight of the plant and probably reflects a relatively small diversion of assimilate to the hyphae (see Table 6, b). This small diversion to the fungus increased phosphate absorption and resulted in a fresh weight increase of 2.77 g (150% increase in plant growth) in the test soil used - a handsome return on the investment! The greater translocation of ^{14}C assimilate to the roots of mycorrhizal plants was due to greater amounts of root in the mycorrhizal system, not to large fungal sinks.

On the evidence of this study we propose sucrose and glucose to be the principal sugars transferred and that low levels of sucrose in the fungal cytoplasm, maintaining a concentration gradient for further passive movement or facilitated diffusion, are achieved by its conversion into glycogen and eventual incorporation into lipid, protein and amino and organic acids involved in the active growth of the fungus (excess acids would be stored in vacuoles).

Both *Endogone* species had high incorporation into the metal-precipitated fraction in common; we do not know if this fraction also serves a storage function (similar to glycogen) and this needs further study. After 24 hr, *E. mosseae* had little label in organic acids and some 20% in polysaccharides whereas *E. araucareae* was quite the reverse with 30% in the acid fraction. Whether these differences are a host induced response rather than a specific fungal characteristic remains to be demonstrated, as organic acids might be expected to play a balancing role in cation uptake by mycelium.

Further work along these lines is warranted as is a definition of the carbohydrate physiology of typical structural features of endomycorrhizas, namely vesicles, arbuscules and chlamydospores. The role of vesicles has yet to be satisfactorily

explained; are they temporary storage organs? If so, are storage compounds in vesicles different from those in hyphae? There is some evidence (Bevege, 1971) that vesicle frequency maximises under conditions of supraoptimal nitrogen supply to the host, but whether they store this excess nitrogen, and if so in what form, is unknown. In our experience vesicles can accumulate a limited quantity of phosphate, but have never been observed to fulfil any infective role and tend to be formed late in the development of the infection (Bevege & Bowen, 1975; Bowen *et al.*, 1975). What storage compounds besides glycogen characterise spores as distinct from the somatic hyphae? The presence of occluded pores in many *Endogone* spores would infer that mature spores are effectively isolated from further material transfer from the body of hyphae.

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